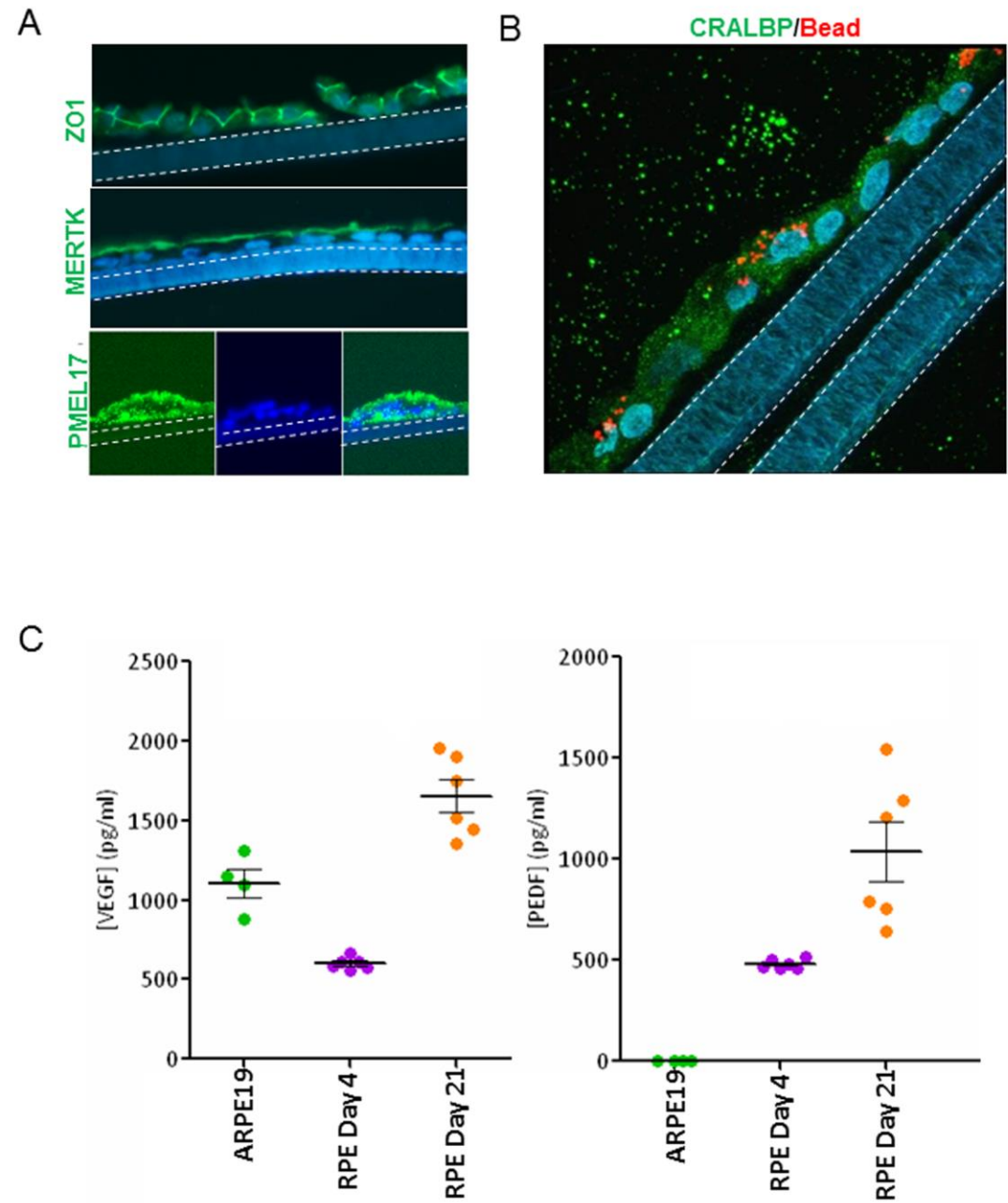


SUPPLEMENTARY FIGURES



## 8 **FigureS1**

9 A. Representative confocal images of X-Z cross-sections of RPE cells cultured on transwells

10 immunostained for ZO1, MERTK and PMEL17 (green). Nuclei are stained with DAPI (blue).

11 Autofluorescence can also be seen from the polyester membrane of the transwell and has been

12 indicated within dotted white lines. Images have been captured at 40x magnification (ZO1, MERTK)

13 or 20x magnification (PMEL17).

14 B. Representative confocal image showing phagocytosis of fluorescent bead (red) by RPE. RPE grown

15 on transwells were incubated with polystyrene beads for a period of 24h at 37°C. The transwell

16 membrane was immunostained for CRALBP (green) to show RPE coverage membrane and sectioned.

17 The beads can be seen within the CRALBP positive area but outside the nuclei stained with DAPI

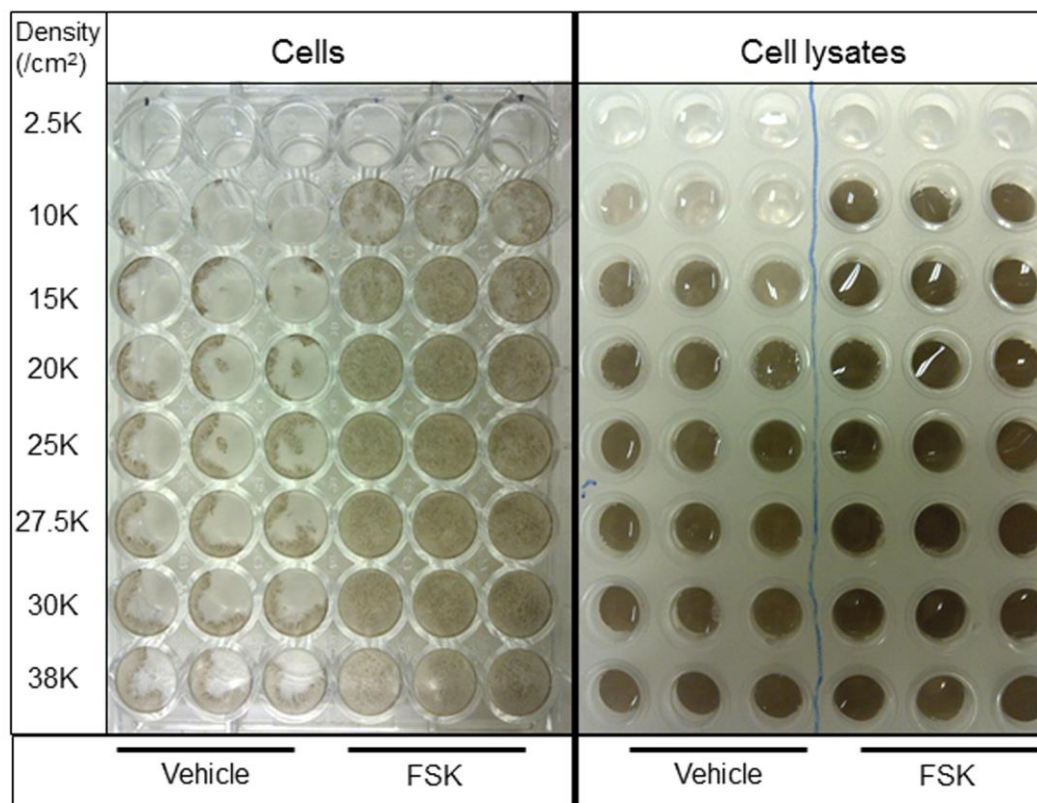
18 (blue) indicating internalization by phagocytosis. Images have been captured at 63x magnification.

19 C. Spent media was collected from RPE and ARPE19 cultures seeded on transwells at Day 4 (RPE) and

20 Day 21 (RPE, ARPE19). VEGF and PEDF were quantified to show that their secretion increases with

21 time in culture. Bars represent Mean $\pm$  SD (n=4-6). P<0.05, Student's t-test.

22



**Figure S2:**

Increasing cAMP signalling promotes acquisition of RPE identity across multiple cell densities.

Increased pigmentation and cell coverage with forskolin treatment are evident in cells and cell

lysates. (K= x1000)

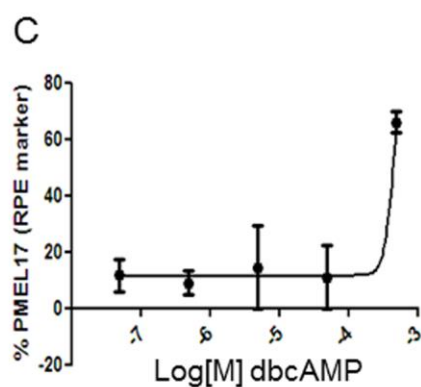
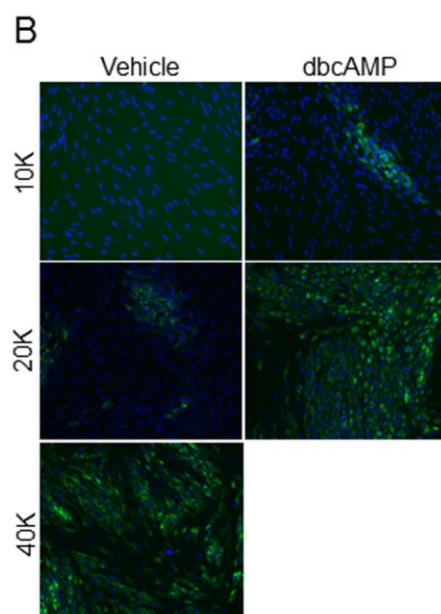
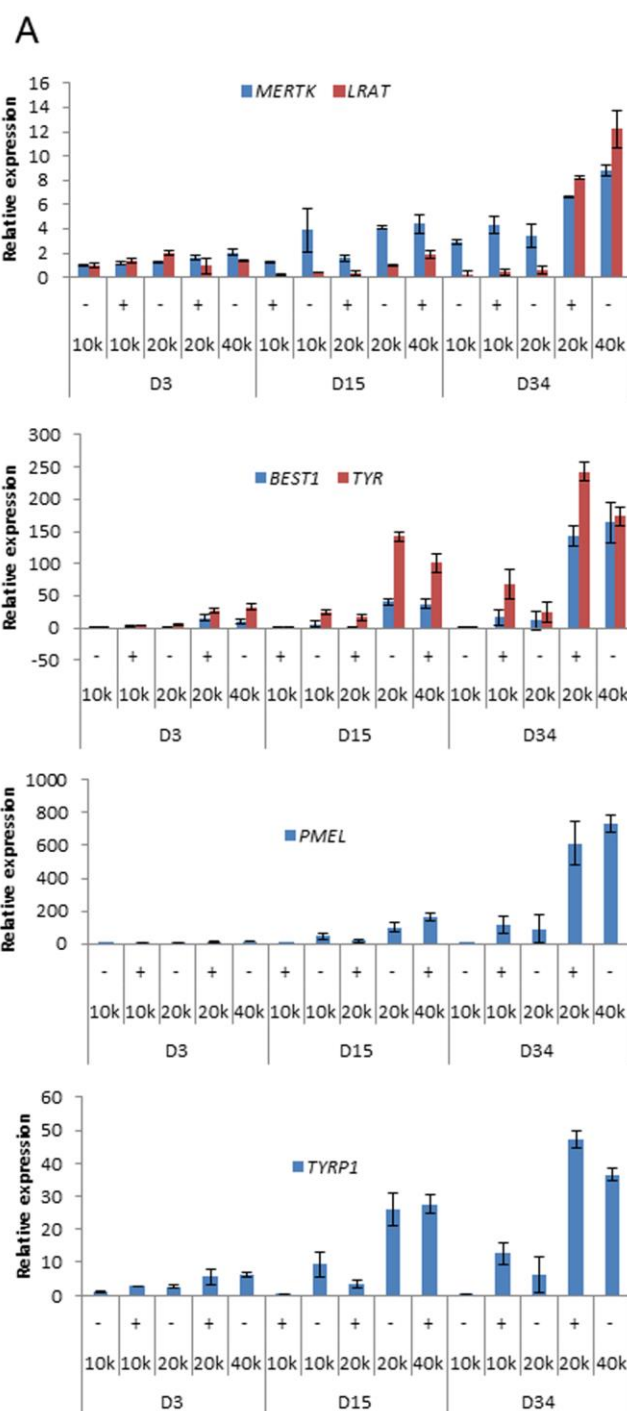


33 **Figure S3:**

34 A. Heatmap showing clustering of expression of epithelial and mesenchymal markers, as measured  
35 by qPCR, where epithelial marker genes cluster together and are distinct from mesenchymal  
36 markers. qPCR data from samples seeded at 38000 cells/cm<sup>2</sup> in media at Day63 in culture were used  
37 to generate this heatmap. Gene specific qPCR showing effect of FSK on expression of epithelial (B, C)  
38 and mesenchymal markers (D, E) across a range of densities at Day63 is shown.

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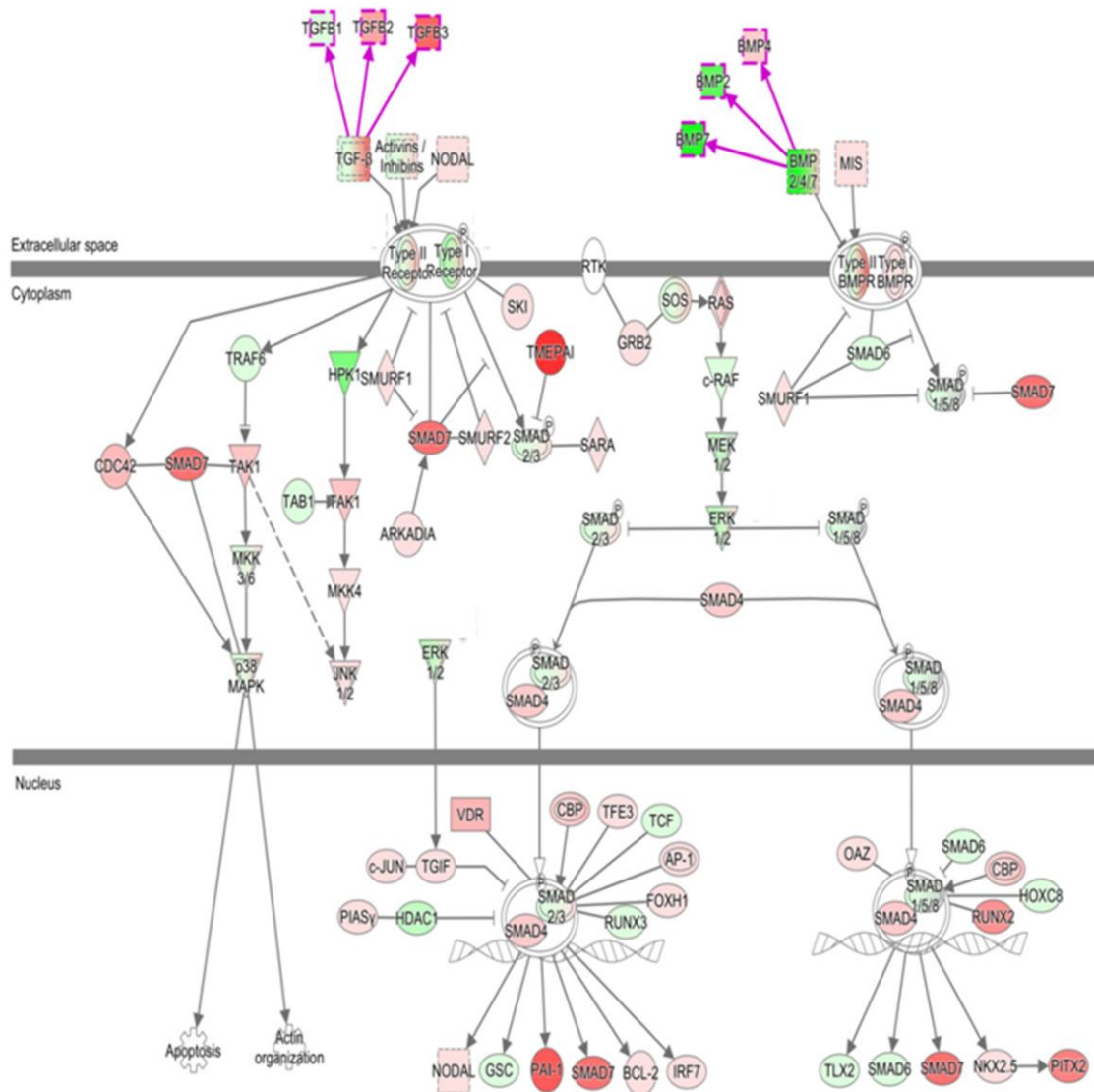
45

46 **Figure S4:**

47 A. qPCR based measurement of transcript expression of a panel of RPE markers in cells seeded at  
48 different densities (10k, 20k, 40k; k=1000 cells/cm<sup>2</sup>) at day 3, 15, 34 (D3, D15, D34) in culture in the  
49 presence (+) or absence (-) of 0.5mM dbcAMP. *GAPDH* and *ACT* are used as a housekeeping genes.  
50 Bars represent Mean  $\pm$  SD (n=3). P<0.05 (Student's t-test).

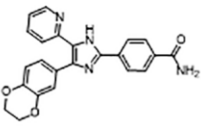
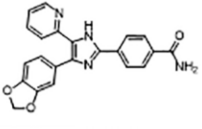
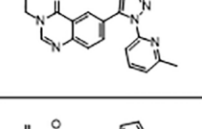
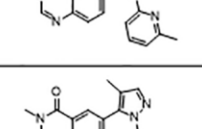
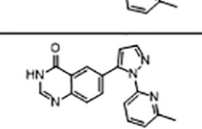
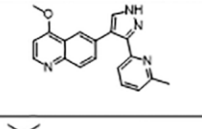
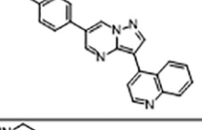
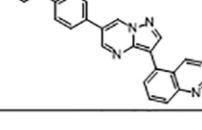

51 B. Representative images showing immunocytochemistry for epithelial marker PMEL17 (green) at  
52 day 15 in cultures seeded as above in the presence or absence of dbcAMP. Nuclei are counterstained  
53 with DAPI (blue). Images have been captured at 10x magnification.

54 C. dbcAMP dose of at least 0.5mM is required to observe effect on RPE phenotype. RPE were seeded  
55 at 20000 cells/cm<sup>2</sup> and treated with different concentrations of dbcAMP for a period of 14 days.  
56 Immunocytochemistry was performed for PMEL17 which showed that a concentration of at least  
57 0.5mM dbcAMP was required to observe an upregulation of this marker of RPE phenotype. (n=3,  
58 Bars=  $\pm$  SD)



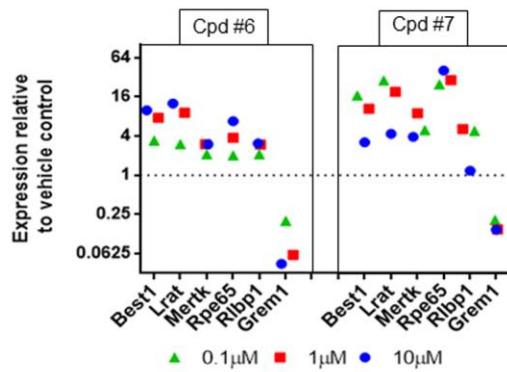
**Figure S5:**

Schematic of the TGF $\beta$  signalling pathway. The downstream signalling effectors are coloured according to their differential expression in high density vs low density cultures. Red indicates decreased expression and green indicates increased expression in high vs. low density cultures with the intensity of the shade indicating fold change. Low expression of TGF $\beta$  and its downstream effectors, as seen by red shading, suggests suppression of the TGF $\beta$  pathway in high density cultures compared to low density cultures.

Cpd #	Structure	ALK5 IC <sub>50</sub>	ALK2 IC <sub>50</sub>	Pmel EC <sub>50</sub>	EdU EC <sub>50</sub>	Reference
1		500nM	n.d.	1.3μM	1.6μM	Rena G et al. EMBO reports. 2004;5:60-65. Callahan JF et al. Journal of medicinal chemistry. 2002;45:999-1001.
2		150nM	22μM	1.7μM	600nM	Compound 14: Rena G et al. EMBO reports. 2004;5:60-65. Surmacz B et al. Stem cells. 2012;30:1875-1884
3		69nM	n.d.	600nM	1.0μM	Compound 10: Boys et al. Bioorganic & medicinal chemistry letters. 2012;22:3392-3397
4		4nM	n.d.	70nM	100nM	Compound 3: Boys et al. Bioorganic & medicinal chemistry letters. 2012;22:3392-3397
5		4nM	n.d.	100nM	100nM	Compound 4: Boys et al. Bioorganic & medicinal chemistry letters. 2012;22:3392-3397
6		1nM	n.d.	1.3μM	100nM	Compound 6: Boys et al. Bioorganic & medicinal chemistry letters. 2012;22:3392-3397
7		Not published	n.d.	100nM	130nM	Patent:WO200426306
8		5.9μM	12.6nM	n.a.	n.a.	DMH1: Mohedas AH et al. ACS chemical biology. 2013;8:1291-1302.
9		9.2μM	1.3nM	n.a.	n.a.	LDN-212854 : Mohedas AH et al. ACS chemical biology. 2013;8:1291-1302.

**Figure S6:**

Table summarizing published IC<sub>50</sub> values against ALK5 and ALK2 for compounds tested [57-61], compound structures and EC<sub>50</sub> values for effect on %PMEL17 and %EdU measured by immunocytochemistry (calculated in this study).



**Figure S7:**

qPCR based measurement of transcript expression of a panel of epithelial (*BEST1*, *LRAT*, *MERTK*, *RPE65*, *RLBP1*) and mesenchymal (*GREM1*) markers in RPE seeded at 15000 cells/cm<sup>2</sup> and treated with Compound #6 and #7 at a concentration of 10μM, 1μM and 0.1μM for a period of 14 days. Data is normalized to expression of vehicle control. *GAPDH* and *HPRT1* are used as housekeeping genes.

## Supplemental Tables

### Table S1:

Table showing list of genes in Cluster 1 and Cluster 2 from Figure 1B.

### Table S2:

Table showing list of GO terms upregulated in the presence of dbcAMP in cultures seeded at 20000 cells/cm<sup>2</sup> at Day 34.